the retroviral vector, said promoter regulating, after infection of the target cell, expression of at least one of the coding sequences being inserted into the body of the vector.

Please add the following claims:

~~31. The retroviral vector according to Claim 1, wherein said regulatory elements and promoters are target cell specific in their expression.~~

32. The retroviral vector according to Claim 5, wherein said regulatory elements and promoters are target cell specific in their expression.

REMARKS

Claim amendments

Claims 8 and 30 have been canceled without prejudice to prosecution of the subject matter claimed in this, related or subsequent applications. Claims 1, 5, 7, 9, 11, 12, 13, 15, 17, 18, 19, 21, 22, 23 and 28 have been amended to more clearly indicate that the retroviral vector which undergoes promoter conversion comprises a 5' long terminal repeat region of the structure U3-R-U5; one or more sequences selected from coding and non-coding sequences; and a 3' long terminal repeat region comprising a partially deleted U3 region wherein said partially deleted U3 region comprises a heterologous "promoter not related to the retroviral vector, said promoter regulating, after infection of the target cell, expression of at least one of the coding sequences being inserted into the body of the vector". Support for the amendment can be found, for example, in the specification on page 9, line 33 - page 10, line 1 and in original Claims 1, 5 and 8. Claims 31 and 32 have been added. Support for newly added Claims 31 and 32 can be found, for example, in original Claim 6 of the subject application.

Priority

As acknowledged by the Examiner, Applicants claim foreign priority to International Application No. PCT/EP95/03445 filed September 1, 1995 and to Danish Application No. 1017/94 filed September 2, 1994 under 35 U.S.C. § 119. Certified copies of International Application No. PCT/EP95/03445 and Danish Application No. 1017/94 are being filed concurrently.

Objection of Claims 22-24 and 30

The Examiner states that Claims 22 and 30 should be amended to recite "The recombinant retroviral particle" (Office Action, page 3). Claim 30 has been canceled. Claim 22 has been amended as suggested by the Examiner.

Rejection of Claims 1, 5, 7-26 and 28-30 under 35 U.S.C. § 112, second paragraph

Claims 1, 5, 7-26 and 28-30 are rejected under 35 U.S.C. § 112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 3).

The Examiner states that Claims 5, 7, 8 and 16 are indefinite because a comma does not appear after the term "promoters" and suggests amending Claim 5 to insert a comma. The term promoters has been deleted from Claim 5, thus obviating the rejection.

The Examiner states that Claim 7 is indefinite because a comma does not appear after the terms "promoters" and "glands" and suggests amending Claim 7 to insert a comma. Claim 7 has been amended to insert a comma after "glands", however, Applicants disagree that a comma should be placed after the term "promoters" in line 9 of Claim 7. The phrase following the term "promoters" (*i.e.*, conferring responsiveness to glucocorticoid hormones or directing expression to the mammary gland) is intended to modify the promoters immediately preceding this phrase (*i.e.*, Mouse Mammary Tumor Virus specific regulatory elements and promoters). Placing a comma after "promoters" could indicate that the phrase modifies all the promoters listed in Claim 7, which would render Claim 7 indefinite.

The Examiner states that Claim 9 is indefinite because a comma does not appear after the term "virus" in line 7 and suggests amending Claim 9 to insert a comma. Claim 9 has been amended as suggested by the Examiner.

The Examiner states that Claims 11 and 12 are indefinite because a comma does not appear after the phrase "cytokine genes" and suggests amending Claim 11 to insert a comma. Claim 11 has been amended as suggested by the Examiner.

The Examiner states that Claim 12 is indefinite because a comma does not appear after the phrase "(HPRT) gene" and suggests amending Claim 12 to insert a comma. Claim 12 has been amended as suggested by the Examiner.

The Examiner states that Claim 21 is indefinite because a comma does not appear after the phrase "regulatory sequences and promoters" and suggests amending Claim 21 to insert a comma. Claim 21 has been amended as suggested by the Examiner.

The Examiner states that Claim 30 should be amended to recite the phrase "with the retroviral vector according to Claim 29". Claim 30 has been canceled, thus, obviating the rejection.

The Examiner states that Claims 18 and 19 should be amended to recite "The kit of Claim 17". Claims 18 and 19 have been amended as suggested by the Examiner.

The Examiner states that Claims 1, 5, 7-26 and 28-30 are indefinite for reciting the phrase "target cell type restricted" because it is not clear to what type of restriction the claims are drawn. The Examiner suggests amending the claims "to be drawn to regulatory elements and promoters that are target cell specific in their expression as in originally presented (now cancelled) claim 6" (Office Action, page 5). The phrase "target cell type restricted" has been deleted from the claims, thus obviating the rejection.

The Examiner states that there is insufficient antecedent basis for the phrase "said target cell specific regulatory elements and promoters" in Claim 7 and suggests amending the claim to recite "wherein the regulatory elements and promoters". Claim 7 has been amended as suggested by the Examiner.

The Examiner states that Claim 13 should be amended to recite "wherein at least one of said coding sequences is an altered or partially deleted retroviral gene". Claim 13 has been amended as suggested by the Examiner.

Rejection of Claims 1, 5, 7-9, 11-13, 16-25 and 28-30 under 35 U.S.C. § 103(a)

The rejection of Claims 1, 5, 7-9, 11-13, 16-25 and 28-30 under 35 U.S.C. § 103(a) "as being unpatentable over Faustinella *et al.* in view of Couture *et al.* in view of Mee *et al.*" (Office Action, page 7) is maintained. It is the Examiner's opinion that:

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vector of Faustinella *et al.* by the use of the LTR regions of Mee et al. because Couture et al. show that insertion of a promoter region in a deleted 3' U3 region of a retroviral vector results in expression of vector genes under the control of the inserted promoter in a cell type specific manner, and that internal promoters may not function properly in a retroviral vector, and that target cell specific retroviral vectors have utility in gene therapy protocols, and because Mee et al. show that their LTR promoter may be used to

> manipulate gene expression in a variety of cell types. It would have been further obvious to use packaging cell lines PA317 and GP&E86 because Couture et al. shows that they may be used to package retroviral vectors (Office Action, page 11).

Applicants respectfully disagree. Applicants teach a retroviral vector wherein the partially deleted 3' U3 region comprises only a heterologous promoter, which is not directly linked to a gene. According to the present invention the heterologous promoter regulates expression of a coding sequence inserted in the body of the vector after infection of the target cell. Thus, the promoter is not directly linked to the coding sequence in Applicants' claimed retroviral vector. The teachings of the cited references, either alone or in combination, do not teach a retroviral vector wherein the U3 region comprises a heterologous promoter which regulates expression of a coding sequence inserted into the body of the vector after infection of the target cell.

Faustinella *et al.* disclose a modified MLV vector comprising a partially deleted 3' U3 region which is substituted by either a luciferase reporter gene operably linked to a rous sarcoma virus promoter or a hygromycin resistance gene operably linked to a herpes simplex thymidine kinase promoter. Alternatively, Faustinella *et al.* teach that the gene operably linked to the promoter can be subcloned into the body of the vector. Notably, these vectors contain a gene directly linked to the promoter in the partially deleted U3 region or in the body of the vector.

Couture *et al.* disclose a modified MLV vector wherein the U3 region is replaced by corresponding U3 regions from closely related murine retroviruses (see Couture *et al.*, Figure 1 and page 671). Couture *et al.* do not disclose a retroviral vector wherein the 3' U3 region comprises a heterologous promoter for any purpose.

Mee *et al.* disclose a Self-Inactivating-Vector (SIN), *i.e.,* a vector with a completely deleted U3 region. Accordingly, the teaching in the Mee *et al.* reference has no relevance to Applicants' claimed vector in which the U3 region is partially deleted and wherein the partially deleted U3 region comprises a heterologous promoter.

As indicated in the previous Amendment mailed to the Patent Office on September 16, 1998, 35 U.S.C. § 103 requires both (1) that the prior art would have suggested to the person of ordinary skill in the art that they should carry out the claimed process and (2) that the prior art establish a reasonable expectation of success (*In re Vaeck,* 20 USPQ2d 1438, 1442 (Fed. Cir. 1991). At most, the combined teachings of Faustinella *et al.*, Couture *et al.* and Mee *et al.* direct the skilled person in the art to insert a gene directly linked to a promoter within the 3' U3 region

or in the body of the vector. In the instance in which a promoter is inserted into the 3' LTR and not directly linked to the gene, the combined teachings direct a person of skill in the art to use a corresponding U3 region from a closely related retrovirus. The combined teachings of the cited references do not teach a retroviral vector wherein the U3 region comprises a heterologous promoter which regulates expression of a coding sequence inserted into the body of the vector after infection of the target cell. Furthermore, the combined teachings in the cited art do not provide a reasonable expectation that doing so would result in expression of the gene.

According to the general state of the art, retroviral vector systems (DNA) comprise identical 5' and 3' LTRs, both having the structure U3-R-U5. For transfer of these DNA constructs into target cells the DNA must at first be transcribed into RNA. Although both LTRs of the DNA constructs are identical in structure, they are distinct in function: the 5' LTR directs initiation of transcription at the R-region of the 5' LTR, while the 3' LTR directs cleavage and polyadenylation of the transcript at the R-region of the 3' LTR (see Figure 3 of the subject disclosure). However, the R-regions of the 5' LTR and the 3' LTR are identical in structure and comprise polyadenylation signals. Nevertheless, transcription reads through the end of the R region of the 5' LTR but stops at the end of the R-region of the 3' LTR.

To explain the apparent inability of the 5' LTR to function efficiently as a poly(A) addition site it is presumed that sequences upstream of the R-region of the 5' LTR, namely in the U3 region, allow transcriptional read-through of the 5' R-region. Accordingly, when developing retroviral vector constructs with an altered U3-region, as disclosed by Faustinella *et al.*, Couture *et al.*, and in the subject disclosure, only the U3-region of the 3' LTR is altered, while the normal 5' U3 region is maintained to ensure transcriptional read-through of the 5' R-region during generation of recombinant retroviral particles. However, the RNA generated only contains the altered 3' U3 regions. A quite different situation is given when working with a SIN construct as disclosed by Mee *et al.* In a SIN vector, the U3-region of the 3' LTR is not altered, but completely deleted, and the normal 5' U3-region is maintained to ensure transcriptional read-through of the 5' region. However, in the SIN vector, the RNA generated does not contain a U3 region.

In the infected target cell the RNA is reverse transcribed into DNA. During reverse transcription, the altered 3' U3 region is duplicated into the 5' LTR. When using a SIN vector, neither the 5' nor the 3' LTR contains a U3 region. Thus, when comparing the constructs described in the Faustinella *et al.*, Couture *et al.* and Mee *et al.* references with the construct

according to the present invention, the following differences occur after integration into the host cell genome:

1) according to Faustinella *et al.*, the partially deleted U3 region upstream of the 5' R region always comprises a gene linked to a promoter; alternatively, the gene linked to the promoter can be inserted into the body of the vector (Figure 2);

2) according to Couture *et al.*, the wild type U3 region upstream of the 5' region is replaced with a corresponding region of closely related retroviruses which drives expression of the gene inserted into the body of the vector (see Figure 1 and whole disclosure of the document); and

3) according to Mee *et al.*, there is no U3 region upstream of the 5' R region in the SIN vector and the promoter + gene are only inserted into the body of the vector.

In contrast, according to the present invention the partially deleted U3 region upstream of the 5' region comprises only a heterologous promoter, not related to the retroviral vector, which drives expression of foreign genes inserted into the body of the vector. Importantly, the coding sequences of the foreign genes are not directly linked to the heterologous, non-related promoter.

After integration into the host cell genome transcription of the genes is expected in the Faustinella *et al.* constructs, the Couture *et al.* constructs, and in the Me *et al.* SIN constructs, but not in the constructs of the present invention.

In Faustinella *et al.* the gene and promoter are always directly linked when inserted into the 5' U3 region and when inserted into the body of the vector. The same applies for a SIN construct according to Mee *et al.* wherein gene and promoter are only inserted into the body of the vector and are also always directly linked. Accordingly, when using such "cassettes", the problem of transcriptional read-through of the 5' R region is circumvented. Since transcription of each gene is independently driven by its "own", directly linked promoter, regulatory elements present in the normal 5' U3 region which allow transcriptional read-through of the 5' R region and which are deleted in the altered (Faustinella *et al.*) or completely deleted (Mee *et al.*) 5' U3 region, are without relevance, because a transcriptional read-through of the 5' R region is no longer needed. In the constructs of Faustinella *et al.* and Mee *et al.* transcription of the genes

occurs independently of sequences upstream of the R region of the original retroviral vector; the vector is only used as a shuttle for transferring expression-cassettes into the genome of the target cell.

In contrast to Faustinella *et al.* and Mee *et al.*, Applicants teach that transcription of foreign genes, which are inserted into the body of the vector and which are not directly linked to a promoter, depends on a heterologous promoter inserted into the 5' U3 region. In this case, transcriptional read-through of the 5' LTR R region for expression of the genes is undoubtedly required. However, as described in the examples of the subject application, the original 5' U3 region is partially deleted up to the inverted repeats. Thus, a person of skill in the art would have expected that the original retroviral vector elements allowing transcriptional read-through of the 5' R region of the retroviral vector had also been deleted, and, therefore, that the heterologous promoter, not directly linked to a gene and inserted into a partially deleted U3 region, could not direct expression of the gene inserted into the body of the vector. Therefore, Applicants' finding that a promoter, which is not related to the retroviral vector and which is inserted into the U3 region of a retroviral vector, regulates expression of a gene inserted into the body of the vector after infection of the target cell is a surprising result.

The same is true when considering the disclosure of Couture *et al.* Couture *et al.* replaced the 5' U3 region with a corresponding region from closely related murine retroviruses. Accordingly, the 5' U3 region of Couture *et al.* is essentially unchanged. Thus, in view of the arguments presented above, it is not surprising to the person skilled in the art that the 5' U3 regions in which only closely related regions and promoters are exchanged still allow transcriptional read-through of the 5' R region.

Based upon the teachings in the cited art and the general state of the art, a skilled practitioner being aware of the transcriptional regulation of genes inserted into the body of a retroviral vector would have only considered, if at all, 1) replacing the wild type promoter of the U3 region with a promoter from a related retrovirus to direct expression of a coding sequence in the body of the vector or 2) inserting a coding sequence directly linked to a heterologous promoter into the U3 region or the body of the vector. However, in view of the above arguments, the skilled practitioner would not have considered inserting a heterologous promoter not related to the retroviral vector into a partially deleted U3 region for the purpose of directing expression of a foreign gene inserted into the body of the vector, because in this case the practitioner would have expected that a transcriptional read-through of the 5' R region, and, thus

expression of the foreign gene would not occur. Clearly, the combined teachings of Faustinella *et al*, Couture *et al.* and Mee *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

Furthermore, in the scientific literature it is reported that genetic rearrangement occurs during reverse transcription, especially when heterologous elements are inserted into the U3 region of the LTR (see Junker, U., *et al., Gene Therapy, 2*:639-646 (1995) which is being filed concurrently as the Exhibit). Such genetic rearrangements are without relevance to the SIN vector of Mee *et al.* and the vector of Couture *et al.* because in both instances no heterologous elements are inserted into the U3 region. As already mentioned above, in the SIN vector of Mee *et al.* the U3 region is completely deleted and in the vector of Couture *et al.* the U3 region is replaced by a U3 region of a closely related virus. In the Faustinella *et al.* vector, genetic rearrangement could occur, however, since the promoter and gene are always directly linked, it is unlikely that genetic rearrangement during reverse transcription would occur in such a way that the promoter no longer drives gene expression.

In contrast, a person skilled in the art would have expected genetic rearrangement to occur in Applicants' claimed vectors which comprise a heterologous promoter inserted into the U3 region. Accordingly, genetic rearrangement could be expected during reverse transcription of Applicants' claimed vectors. Since the promoter additionally drives gene expression of genes not directly linked to the promoter, but inserted into the body of the vector, it could also be expected that genetic rearrangement results in constructs in which the promoter is no longer placed upstream of the genes. Thus, a person of skill in the art would not expect gene expression to occur after integration of Applicants' construct into the host cell genome. However, Applicants unexpectedly found that their claimed vector remains stable and that foreign genes inserted into the body of the vector are expressed.

As discussed above, the combined teachings of Faustinella *et al.*, Couture *et al.* and Mee *et al.* do not teach a retroviral vector wherein the U3 region comprises a heterologous promoter which regulates expression of a coding sequence inserted into the body of the vector after infection of the target cell. Furthermore, it is clear from the knowledge in the art at the time of Applicant's invention that, based on the combined teachings of the cited art, the skilled practitioner would not expect that the heterologous promoter of Applicants' claimed retroviral vector would direct expression of a coding sequence in the body of the vector.

Clearly, the combined teachings of the cited art do not render obvious Applicants' claimed invention, particularly as amended. Withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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